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Ink Jet printing of mammalian primary cells for tissue engineering applications

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ABSTRACT

A piezoelectric drop on demand printer has been used to print primary human osteoblast and bovine chondrocyte cells. After deposition the cells were incubated at 37°C and characterised using optical microscopy, SEM and cell viability assays. Cells showed a robust response to printing exhibiting signs of proliferation and spreading. Increasing the drop velocity results in a reduced cell survival and proliferation rates but both cell types grew to confluence after printing under all conditions studied.

INTRODUCTION

Free form fabrication techniques such as stereolithography, fused deposition modelling and three dimensional ink jet printing are capable of manufacturing scaffolds for use in tissue engineering. These techniques can fabricate scaffolds with the precision required to incorporate a complex interconnected internal architecture and the ability to tailor the structure to both the application and the individual [1-3]. The incorporation of cells into these scaffolds however still poses a significant problem. Current cell seeding techniques, whether static or dynamic, can result in non-uniform distribution, limited penetration depth and utilises a limited variety of cell types [4-5]. It is therefore proposed that ink jet printing be used to simultaneously deposit cells and scaffold materials. Ink jet printing offers the potential of seeding cells deep within the core of a scaffold whilst controlling their location relative to scaffold architecture, varying concentrations and cell types and maximising the use of growth factors.

Xu et al have already demonstrated that cell aggregates remain viable after printing using a modified thermal ink jet printer [6]. Previous work by the author has demonstrated the viability of fibroblasts printed using a piezoelectric ink jet printer [7]. Here we report a study that begins the systematic investigation into cell viability after printing using piezoelectric ink jet technology.

EXPERIMENTAL DETAILS

Human primary osteoblasts were isolated from femoral heads after total hip replacement surgery. Bovine chondrocytes were isolated from metacarpalphalangeal joints obtained from a local abattoir. Cells were harvested using a standard trypsination procedure. The harvested cells were re-suspended in the required quantity of DMEM (dulbeccos modified eagles medium) containing 10% foetal bovine serum, 1% Penicillin & Streptomycin and 0.005% ascorbic acid. To ensure uniform distribution and to disrupt cell clumps the final solution was gently agitated using a pipette.

Printing experiments were carried out using single nozzle tubular piezoelectric jets (Microfab Inc., Plano TX, USA). The nozzle orifice diameters used 60 micron for

both osteoblast and chondrocyte suspensions. All suspensions were printed at a rate of 10000 droplets per second, with piezoelectric driving voltages ranging from 30 to 80 V onto well plates. The latter were standard tissue culture plastics made of non-pyrogenic polystyrene (Costar®). Printed cells were then incubated at 37°C, 5% CO_2 in a standard cell culture laboratory.

The cells were monitored for signs of viability using a standard light microscope. The effect of voltage on cell division was measured using a WST-1 cell proliferation assay (Roche Diagnostics). A Philips XL30 field emission gun scanning electron microscope (Phillips, Netherlands) was used to assess the initial attachment and spreading of printed chondrocytes.

Printed samples of both cell types were monitored using a light microscope over a period of 6 days to ascertain initial cell viability. WST-1 cell proliferation assays were performed on samples printed into a 96 well plate at time points of 4, 24, 48 and 72 hours. In each case cells were allowed to attach before the media was replaced with a known quantity and the WST-1 reagent added. 3 – 6 Repetitions were used within one run for both cell types. Sample absorbance was measured after 4hours incubation with the WST-1 reagent using an Ascent colorimetric plate reader at 450nm (Labsystems, Finland). Chondrocytes were printed onto glass coverslips and processed for SEM analysis using a standard ethanol dehydration procedure. The samples were then sputter coated and viewed using scanning electron microscopy.

DISCUSSION

Drop velocity and mass were measured over a voltage range of 30-70V. Droplet momentum was then calculated from these values and can be seen in figure 1. Increasing the voltage was found to increase the droplet mass, velocity and momentum. This is an important point, as cells printed at higher voltages will be subjected to a higher impulse.

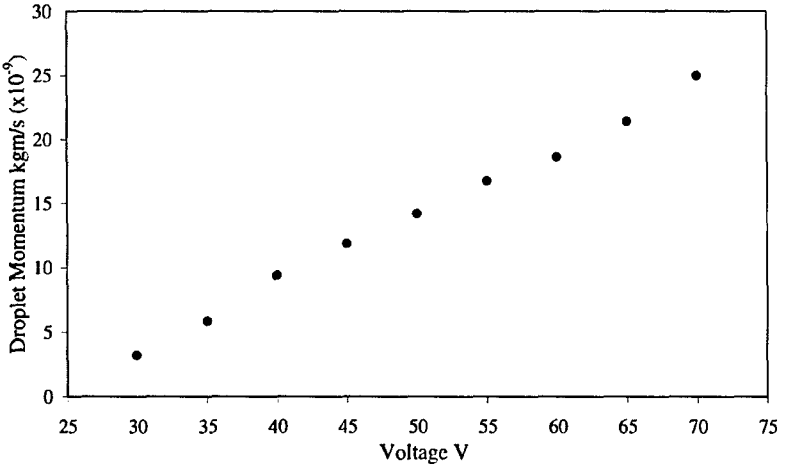

Figure 1) Graph showing the effect of voltage on droplet momentum

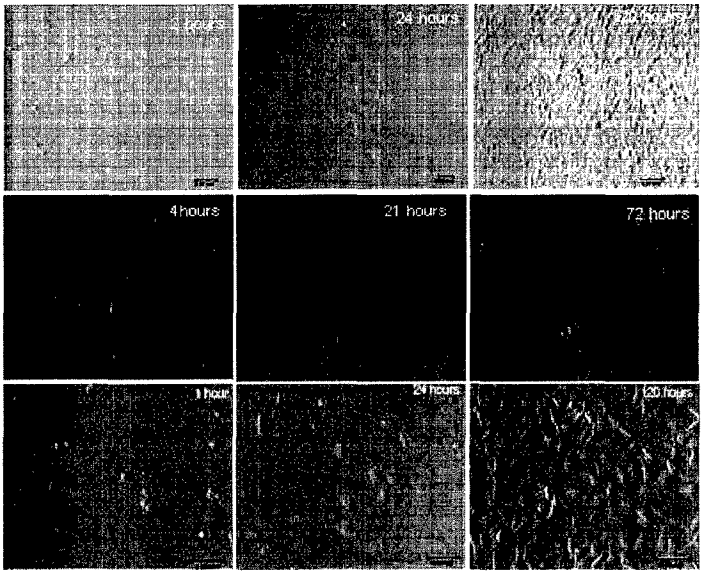

Figure 2) Montage of results of bovine chondrocytes (above, 0.07mm scale bar), primary human osteoblasts (middle, 0.05mm scale bar), fibroblasts type II (below, 0.09mm scale bar [7]) after ink-jet printing

Figure 2 shows a montage of light micrographs taken at various time points after printing. Both the primary osteoblasts and the bovine chondrocytes proliferate to confluence as previously observed with printed fibroblasts [7]. Evidence of spreading and attachment can be observed after 24 hours for all cell types.

Preliminary results from the WST-1 cell proliferation assay of osteoblasts (figure 3) shows that lower printing voltages result in a higher cell proliferation rate. The proliferation rate can be affected by the initial cell seeding density which should decrease with the voltage due to smaller drop volumes. This assay highlighted the need for a longer printing time to generate reliable results at a 4 hour time point as such the printing time was increased from 10s to 30s for the chondrocyte samples. Cell proliferation data for Chondrocytes printed at 35, 40, 60 and 80V over 30s is more informative (figure 4). The cell proliferation rate between 35 and 40V is identical with a marked difference between initial cell seeding which can be attributed to the smaller droplets generated by the lower voltage. The 60V sample demonstrated an increase in cell number until a decline at 72 hours this is attributed to the attainment of confluency resulting in cell death. Chondrocytes printed at 80V declined in number over the initial 24 hours suggesting that cell death as a result of printing occurs. It is speculated that this death is due to mechanical damage to the cell membrane due to the increased impulse forces as remaining viable cells continued to proliferate over the remaining culture times.

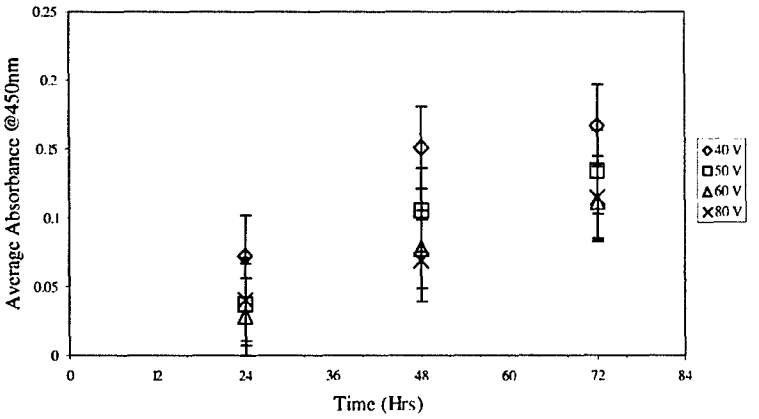

Figure 3) WST-1 cell proliferation results for osteoblasts at 24, 48 and 72 hours after printing at a variety of voltages. Nominal error bars calculated from a small set of sample data.

The observations between the 60V and 80V samples are supported by SEM micrographs taken after 24 hours (figure 5). Chondrocytes printed at 60V show a high degree of spreading and attachments whilst those printed at 80V have attached but show little signs of spreading.

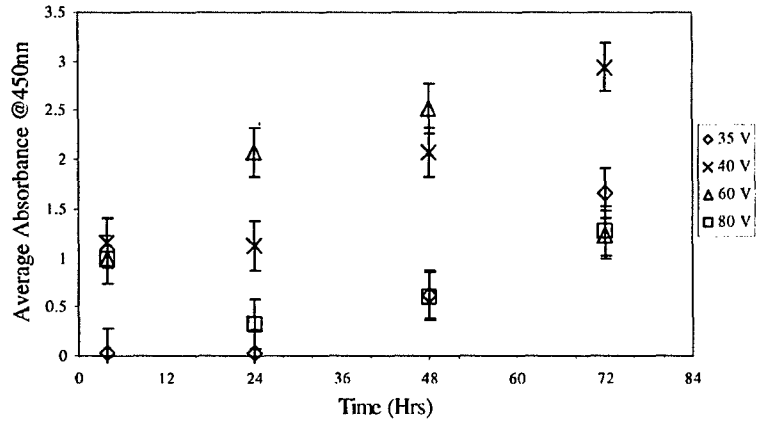

Figure 4) WST-1 cell proliferation results for chondrocytes at 4, 24, 48 and 72 hours after printing at a variety of voltages. Nominal error bars calculated from a small set of sample data.

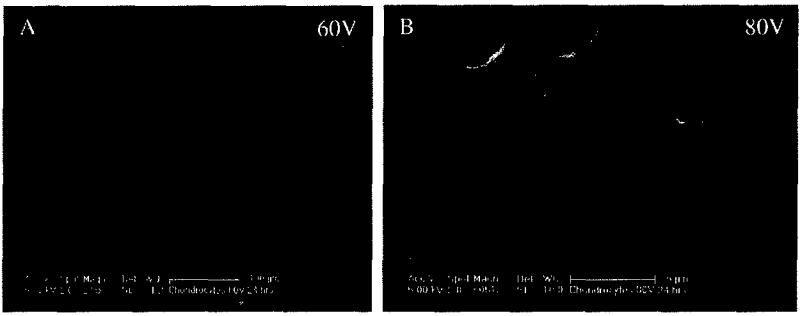

Figure 5) SEM micrographs of chondrocytes 24hours after printing at A) 60V and B) 80V

CONCLUSION

It was determined that increasing the printing voltage increased droplet mass, velocity and momentum. Therefore cells printed at 80V will be subjected to higher impact forces than those printed at lower voltages. Monitoring of printed samples using a light microscope showed both cell types attaching, spreading and proliferating to confluence over time. Cell proliferation data indicates that there is a link between increasing the printing voltage and decreased cell proliferation over time. This data highlighted the need for increased sample printing time and resulting in chondrocyte samples being printed over 30s instead of 10s. Chondrocytes printed at 80V showed evidence of cell death over the first 24 hours, which is supported by SEM micrographs showing reduced spreading as compared to a 60V sample at the same time period. However cells remaining after this initial period continued to proliferate at a satisfactory rate. In conclusion it was found that cells can survive the printing process through a wide range of voltages and further work in the 60-80V range should be carried out to ascertain the point at which the printing voltage becomes a limiting parameter. Future work includes quantification of cell death and repetition of the experiments conducted here to confirm these findings. Investigation into ascertaining the mechanism of cell death is also planned.

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